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Development of a Standardized Susceptibility Test for *Campylobacter* with Quality-Control Ranges for Ciprofloxacin, Doxycycline, Erythromycin, Gentamicin, and Meropenem

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ABSTRACT

A standardized agar dilution susceptibility testing method was developed for *Campylobacter* that consisted of testing on Mueller–Hinton medium supplemented with 5% defibrinated sheep blood in an atmosphere of 10% CO_2, 5% O_2, and 85% N_2. *Campylobacter jejuni* ATCC 33560 was identified as a quality-control (QC) strain. Minimal inhibitory concentration (MIC) QC ranges were determined for two incubation time/temperature combinations: 36°C for 48 hr and 42°C for 24 hr. Quality-control ranges were determined for ciprofloxacin, doxycycline, erythromycin, gentamicin, and meropenem. For all antimicrobial agents tested at both temperatures, 95–100% of the QC MIC results fell within recommended QC ranges. Twenty-one *Campylobacter* clinical isolates, encompassing five species of *Campylobacter* (*C. jejuni*, *C. coli*, *C. jejuni*, subsp. *doylei*, *C. fetus*, and *C. lari*) were tested in conjunction with the *C. jejuni* QC strain. While *C. jejuni* and *C. coli* could be reliably tested under both test conditions, growth of *C. jejuni* subsp. *doylei*, *C. fetus*, and *C. lari* isolates was inconsistent when incubated at 42°C. Therefore, it is recommended that these species only be tested at 36°C.

INTRODUCTION

BACTERIA BELONGING TO THE GENUS *Campylobacter* are a leading cause of bacterial gastroenteritis in humans,[3] with *Campylobacter jejuni* and *Campylobacter coli* being the most commonly isolated species. In developed countries, the majority of sporadic cases of *Campylobacter* infection have been linked to the consumption or mishandling of raw or undercooked poultry meat products.[16,23] Surveillance data from the United States[2,5,25] and Europe[6,12,15] indicate that 50–80% of raw retail chicken meats may be contaminated with *Campylobacter*.

Campylobacter enteritis is typically a self-limiting diarrhea that may be indistinguishable from enteritis caused by other intestinal bacterial pathogens such as *Salmonella* and *Escherichia coli*. Although the majority of cases of campylobacteriosis are self-limiting, infections may develop into severe invasive or relapsing disease. The treatment of choice has been erythromycin

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or ciprofloxacin. Traditionally, results from standardized *in vitro* antimicrobial susceptility testing have provided clinicians with insight as to potentially effective antimicrobial agents.[7] Because of the fastidious growth requirements of *Campylobacter*, they cannot be tested reliably using susceptibility testing methods currently available for rapidly growing organisms such as the Enterobacteriaceae or facultative Gram-positive bacteria.

The reported increased incidence of resistance in *Campylobacter*[8,17,23] underscores the need for a standardized susceptibility testing method for organisms in this genus. The object of this study was to develop a standardized testing method in accordance with the guidelines established by the National Committee for Clinical Laboratory Standards (NCCLS).

Following the consensus of the NCCLS Antimicrobial Susceptibility Testing (AST) committee, the goal of the multicenter study described here was to establish quality-control (QC) ranges (MICs) for those antimicrobial agents most likely to be used for the treatment of campylobacteriosis: ciprofloxacin, doxycycline, gentamicin, erythromycin, and meropenem. The AST committee also recommended that two incubation times and temperatures be investigated, 42°C for 24 hr and 36°C for 48 hr. In accordance with NCCLS guidelines for developing standardized testing methods,[19] we also tested a number of clinical isolates of *Campylobacter*, consisting of five *C. jejuni*, five *C. coli*, five *C. jejuni* subsp. *doylei*, three *C. fetus*, and three *C. lari* to verify that the method is appropriate for testing *Campylobacter* clinical isolates.

MATERIALS AND METHODS

Participating laboratories

The data presented here were generated in a multilaboratory studies in accordance with the guidelines described in the NCCLS M23-A5 document. These laboratories included: Michigan State University, College of Medicine (East Lansing, MI); Clinical Microbiology Institute (Wilsonville, OR); Focus Technologies (Herndon, VA); Duke University Medical Center (Durham, NC); The Food and Drug Administration, Center for Veterinary Medicine, Office of Research (Laurel, MD); Danish Veterinary Institute (Copenhagen, Denmark); Division of Immunity and Infection, The Medical School, University of Birmingham (Birmingham, UK); University of Alberta Hospital (Edmonton, Alberta, Canada); and Abbott Laboratories (Abbott Park, IL).

Bacterial strains and growth conditions

In preliminary studies [not reported here, but accepted by the NCCLS Subcommittes on AST and Veterinary Antimicrobial Susceptibility Testing (VAST)], a number of growth parameters and testing methodologies were examined that resulted in agar dilution as the reference testing method, *C. jejuni* ATCC 33560 as the QC organism, Mueller–Hinton (MH) agar supplemented with 5% defibrinated sheep blood as the growth medium, and growth in a 10% CO_2, 5% O_2 atmosphere. *C. jejuni* ATCC 33560 was selected as the QC organism based on *in vitro* growth characteristics consistent with those of clinical isolates, stability in its antibiogram following multiple passages on artificial medium and following long-term storage, MIC ranges similar to those observed for clinical isolates, and intra- and interlaboratory MIC reproducibility. Susceptible human clinical isolates of *C. jejuni*, *C. jejuni*, subsp. *doylei*, *C. coli*, *C. lari*, and *C. fetus* were generously provided by M.J. Ferraro (Department of Clinical Microbiology, Massachusetts General Hospital, Boston, MA). A total of five *C. jejuni*, five *C. coli*, five *C. jejuni* subsp. *doylei*, three *C. fetus*, and three *C. lari* were tested in parallel with the QC strain. The number of clinical strains was selected based on the capacity of the manual replicators used in agar dilution, and the need to test 10 replicates of the QC strain. Strains were shipped overnight at ambient temperature in tryptic soy agar (TSA) stabs. Upon receipt by the laboratory, all clinical isolates were stored at −70°C in *Brucella* broth with 20% glycerol until needed. Test isolates were recovered from freezer stocks by overnight incubation in a microaerophilic atmosphere on TSA blood agar plates. Testing was performed on commercially prepared MH agar supplemented with 5% defibrinated sheep blood (PML Microbiologicals, Wilsonville, OR). MIC results were determined following incubation for 36°C for 48 hr or 42°C for 24 hr in an atmosphere containing 5% O_2, 10% CO_2, and 85% N using either Campy pouches (Beckton Dickinson Diagnostic Systems [BDDS] Sparks, MD) or gas-regulated incubators.

Antimicrobial agents

The antimicrobial agents, and the two-fold dilution ranges tested for each drug, were: ciprofloxacin (0.015–8 μg/ml), doxycycline (0.06–32 μg/ml), erythromycin (0.125–64 μg/ml), gentamicin (0.06–32 μg/ml), and meropenem (0.001–0.5 μg/ml). Antimicrobial agents were provided in dehydrated form to the participating laboratories by PML Microbiologicals. The antimicrobial agents were weighed and diluted in accordance with the method described in the NCCLS M7[20] and M31[21] documents.

Agar dilution susceptibility testing method

The study was performed following the guidelines in the NCCLS M23-A2 document.[19] Ten independently prepared replicates of *C. jejuni* ATCC 33560 and one replicate of each of the 21 human isolates of *Campylobacter* were tested daily for 2 days using the described agar dilution method.[18] In accordance with NCCLS guidelines, all isolates were tested on three different lots of MH agar (BDDS lot # 1065000, Remel lot # 169458, Oxoid lot # 22970) per day in each of the participating laboratories. The MH agar media was prepared by a commercial laboratory and sent to the participating laboratory in the form of 17-ml agar deeps contained in 25 × 150-mm screw-capped tubes. On the day of use, the agar deeps were liquified and cooled to 46–48°C. To each deep, a 2-ml aliquots of an antimicrobial dilution were added along with 1 ml of defibrinated sheep blood. The tube was inverted several times to ensure adequate mixing without excessive bubbling, and poured into a 100 × 15-mm petri dish.

Inocula were prepared from overnight growth on blood agar plates by suspending each culture in sterile distilled water or MH broth to obtain a turbidity equivalent to that of a 0.5 McFarland standard. The suspension was added to the wells of a replicator, and replica plating was done using 1-, 2-, or 3-mm

replicating pins, depending on the participating laboratory's capabilities. Laboratories using replicators with 2-mm pins or 3-mm pins diluted the cell suspension 1:10 dilution prior to plating to ensure that the final inoculum size on the agar surface was approximately 1×10^4 colony-forming units (CFU). An inoculation control plate was included at the start and end of each dilution series for each antimicrobial. The control plate consisted of MH agar, 1 ml of defibrinated sheep blood, and 2 ml of sterile water. Plates were allowed to dry on the bench top to allow the inoculum to be absorbed by the agar prior to incubation. This usually required no more than 30 min.

In each laboratory, isolates were tested in parallel at 36°C for 48 hr and 42°C for 24 hr. Inoculated agar plates were inverted and stacked no more that four high to ensure a uniform temperature throughout the incubation period.

RESULTS

Quality-control ranges

Although nine participating laboratories were enrolled in the study, the QC ranges presented here were calculated from data acquired at seven testing sites. Two laboratories deviated from the protocol, resulting in data that were noticably inconsistent with the data from the other seven laboratories.

The MIC results for the QC organism *C. jejuni* ATCC 33560, when tested against the five antimicrobial agents, demonstrated intra- and interlaboratory reproducibility (Table 1). The QC ranges included the observed modal MIC ± 1 $\log_2$ dilution for doxycycline and gentamicin at 36°C/48 hr, erythromycin at 42°C/24 hr, and for meropenem in both incubation conditions. The remaining test conditions resulted in QC ranges $\pm$ 2 $\log_2$ dilutions of the observed modal MIC. For all drugs except gentamicin and meropenem at 36°C, the MIC QC limits encompassed more than 99% of the observed values under both incubation conditions. For gentamicin tested at 42°C/24 hr, 95% of the observed MIC values fell within a bimodal 4 $\log_2$ QC range for this drug.

The QC ranges differed slightly between the two incubation settings, but followed no consistent pattern. For example, doxycycline and gentamicin both showed a 3 $\log_2$ QC range at 36°C/48 hr and a 4 $\log_2$ range at 42°C/24 hr. The reverse was true for erythromycin. Ciprofloxacin and meropenem displayed 4 $\log_2$ and 3 $\log_2$ QC ranges, respectively, in both incubation conditions. Neither was there a clear higher or lower MIC range in the different QC limits relative to each growth temperature (Table 1). For example, the ciprofloxacin QC limits were one dilution lower at 42°C (0.006–0.5 μg/ml *vs*. 0.12–1 μg/ml), whereas, the meropenem limits were one dilution higher at 42°C (0.008–0.03 μg/ml *vs*. 0.004–0.015 μg/ml). Reproducibility at either temperature was not affected by different lots of medium (data not shown).

Clinical isolates

In addition to the main goal of this study, which was to establish a standardized testing method, we tested 21 human clinical isolates of *Campylobacter* to demonstrate that this testing method was applicable to this population of organisms. The data obtained with representative isolates under both incubation conditions are shown in Tables 2 and 3. Although there was a wider range of MIC results compared to that observed for the QC strain, the MIC values of the clinical isolates clustered near the QC ranges for each antimicrobial agent. *C. jejuni* and *C. coli* displayed the best overall reproducibility at both incubation temperatures. For *C. jejuni* subsp. *doylei*, *C. fetus*, and *C. lari*, there were instances where the isolates failed to grow at 42°C (Table 3). In addition, among those isolates for which MIC values were obtained at 42°C, variability in the MIC distribution was greater than that observed for these three species at 36°C.

DISCUSSION

The results of the collaborative study presented here were developed in seven laboratories, in accordance with the NCCLS guidelines described in the M37-A2 document. Following the recommendation of the NCCLS-AST subcommittee, we performed the tests at both 36°C for 48 hr and 42°C for 24 hr incubation conditions. The results of this study have been presented to the NCCLS AST and VAST subcommittees, which accepted the QC organism, testing conditions, and QC ranges for five antimicrobials recommended for the treatment of human campylobacteriosis—ciprofloxacin, doxycycline, gentamicin, erythromycin, and meropenem.

The MIC results with the QC strain *C. jejuni* ATCC 33560 were reproducible within and between laboratories. The MIC values were within a three to four $\log_2$ dilution range with both incubation conditions. For all antimicrobial agents at both temperatures, 95–100% of the QC MIC results fell within recommended QC ranges. No lot-to-lot medium effects were observed. A noteworthy finding of this study is different QC ranges should be applied depending on the incubation temperature used for testing (Table 1).

Clinical isolates of *Campylobacter* were tested to see if the proposed method was applicable to clinical situations. Because *C. jejuni* and *C. coli* are both thermotolerant, clinical isolates of *Campylobacter* are routinely cultured at 42°C to enhance the selective process. Once isolated and in pure culture, growth at higher temperature is not necessary to propagate the organism. For this reason, the initial QC studies involved incubation at 36°C for 48 hr. However, there was concern that the lower temperature would delay the reporting of results by 24 hr. Therefore, incubation at 42°C for 24 hr was also included.

Overall, the MIC values for the clinical isolates clustered around the QC ranges (Tables 2 and 3). In contrast to the results obtained for the QC strain, in which nearly all the MIC values fell within four $\log_2$ dilutions, the clinical isolates of *Campylobacter* showed a wider range of MIC values to some antimicrobials, both within and between laboratories (data not shown). *C. jejuni* and *C. coli* results were most consistent. In addition, comparison of the two testing conditions showed that only *C. jejuni* and *C. coli* gave consistent growth at the higher temperature. For example, when testing *C. jejuni* or *C. coli* against ciprofloxacin at 42°C for 24 hr, all of the isolates produced an MIC end point. However, when tested against ciprofloxacin, growth failure rates were: *C. lari* 30%, *C. jejuni* subsp. *doylei* 23%, and *C. fetus* 13%. Thus, while *C. jejuni* and *C. coli* can be reliably tested at either temperature, the lack of

TABLE 1. AGAR DILUTION QC RESULTS FOR *C. jejuni* ATCC 33560

Antimicrobial agent	*Incubation temperature*	*MIC (μg/ml)*																*% MICs*	
		0.001	*0.002*	*0.004*	*0.008*	*0.015*	*0.03*	*0.06*	*0.12*	*0.25*	*0.5*	*1*	*2*	*4*	*8*	*16*	*32*	*64*	*At mode*	*Within QC range*
Ciprofloxacin																				
	36°C					0	0	0	**5**	**177**	**238**	**0**	0	0	0				57	100
	42°C					0	0	**0**	**148**	**239**	**33**	0	0	0	0				57	100
Doxycycline																				
	36°C							0	0	0	**76**	**298**	**45**	1	0	0	0		71	99
	42°C							0	0	**0**	**172**	**236**	**12**	0	0	0	0		56	100
Erythromycin																				
	36°C								0	0	0	**73**	**168**	**169**	**10**	0	0	0	80	100
	42°C								0	0	0	**108**	**203**	**109**	0	0	0	0	48	100
Gentamicin																				
	36°C							0	0	0	**43**	**309**	**68**	0	0	0	0		74	100
	42°C							0	10	10	**72**	**258**	**13**	**57**	0	0	0		61	95
Meropenem																				
	36°C	0	10	**88**	**220**	**100**	1	1	0	0	0								52	97
	42°C	0	0	0	**73**	**294**	**53**	0	0	0	0								70	100

Recommended QC ranges are shown in bold type.
Incubation time was 48 hr when incubated at 36°C and 24 h when incubated at 42°C.
Results represent 420 data points per drug (10 suspensions × 3 medium lots × 2 testing days × 7 laboratories).
Comparison of results between laboratories and medium lots are not shown.

TABLE 2. AGAR DILUTION MIC (μG/ML) FOR *Campylobacter* CLINICAL ISOLATES AT 36°C, 48 HR

Antimicrobial agent/ Campylobacter *spp.*	*MIC (μg/ml)*																	*NG/ CTM*	*% MICs*	
	0.001	*0.002*	*0.004*	*0.008*	*0.015*	*0.03*	*0.06*	*0.12*	*0.25*	*0.5*	*1*	*2*	*4*	*8*	*16*	*32*	*64*		*At mode*	*Within QC range*
Ciprofloxacin																				
C. jejuni					0	4	9	**59**	**70**	**54**	**6**	1	0	0				7/0	33	90
C. coli					0	2	24	**56**	**74**	**39**	**12**	0	3	0					35	86
C. doylei					5[a]	26	68	**51**	**34**	**13**	**6**	1	1	1				4/0	32	50
C. fetus					2[a]	0	0	**0**	**4**	**19**	**46**	28	16	0				0/5	38	58
C. lari					4[a]	0	0	**3**	**12**	**9**	**16**	13	21	33[b]				10/5	17	32
Doxycycline																				
C. jejuni							21[a]	50	33	**52**	**31**	**11**	0	0	0	0		10/2	25	45
C. coli							8[a]	64	24	**30**	**33**	**23**	13	8	1	0		0/6	30	41
C. doylei							44[a]	52	30	**24**	**32**	**11**	0	2	2	5[b]		7/1	25	32
C. fetus							1[a]	1	7	**20**	**18**	**17**	14	2	15	17[b]		1/7	17	46
C. lari							21[a]	22	31	**24**	**9**	**2**	0	0	0	0		14/3	25	28
Erythromycin																				
C. jejuni								0	4	16	**65**	**69**	**44**	**4**	0	0	0	5/3	33	87
C. coli								1[a]	18	46	**29**	**34**	**59**	**17**	2	0	0	1/3	28	66
C. doylei								44[a]	71	45	**21**	**20**	**2**	**0**	0	0	0	6/1	34	20
C. fetus								0	0	4	**40**	**34**	**29**	**8**	0	0	0	0/5	33	93
C. lari								6[a]	2	23	**34**	**34**	**14**	**0**	0	0	0	11/2	54	65
Gentamicin																				
C. jejuni							0	5	19	**92**	**73**	**20**	0	0	0	0		0/1	44	88
C. coli							0	3	8	**120**	**69**	**0**	0	0	0	0		1/9	57	90
C. doylei							23[a]	20	96	**24**	**38**	**1**	0	0	0	0		8/0	46	30
C. fetus							3[a]	3	4	**17**	**75**	**12**	0	0	0	0		0/6	63	87
C. lari							9[a]	2	2	**7**	**51**	**37**	6	0	0	0		11/1	40	75
Meropenem																				
C. jejuni	9[a]	10	**20**	**79**	**73**	11	0	1	0	0[b]								6/1	38	82
C. coli	2[a]	2	**5**	**23**	**26**	74	53	19	0	4[b]								2/0	35	26
C. doylei	18[a]	4	**2**	**30**	**49**	64	29	2	1	3								7/1	30	39
C. fetus	1[a]	0	**0**	**0**	**0**	9	66	19	5	15[b]								1/4	55	0
C. lari	16[a]	7	**8**	**21**	**20**	11	6	2	2	14[b]								19/0	33	39

C. jejuni, C. coli, and *C. doylei* represent 210 data points per drug (5 isolates × 3 medium lots × 2 testing days × 7 labs); *C. fetus* represents 120 data points per drug (2 isolates × 3 medium lots × 2 testing days × 7 labs and 1 isolate × 3 medium lots × 2 testing days × 6 labs). *C. lani* represents 126 data points per drug (3 isolates × 3 medium lots × 2 testing days × 7 labs).

NG/CTM, No Growth/Contaminated.

[a]Results represent equal to or less than values.

[b]Results represent equal to or greater than values.

TABLE 3. AGAR DILUTION MIC (μG/ML) FOR *Campylobacter* CLINICAL ISOLATES AT 42°C, 24 HR

Antimicrobial agent/ Campylobacter *spp.*	*MIC (μg/ml)*																*NG/ CTM*	*% MICs*	
	0.001	*0.002*	*0.004*	*0.008*	*0.015*	*0.03*	*0.06*	*0.12*	*0.25*	*0.5*	*1*	*2*	*4*	*8*	*16*	*32*	*64*		*At mode*	*Within QC range*
Ciprofloxacin																				
C. jejuni					0	0	**14**	**80**	**93**	**23**	0	0	0	0					44	100
C. coli					0	0	**20**	**82**	**74**	**24**	8	2	0	0					39	95
C. doylei					7[a]	23	**44**	**44**	**21**	**3**	0	0	0	0				49/19	42	53
C. fetus					1	5	**4**	**17**	**10**	**27**	29	6	2	0				16/3	24	48
C. lari					5[a]	5	**12**	**22**	**4**	**2**	3	1	7	27[b]				38/0	21	32
Doxycycline																				
C. jejuni							9[a]	58	**58**	**51**	**30**	**3**	0	0	0	0		1/0	55	68
C. coli							9[a]	70	**31**	**44**	**31**	**20**	5	0	0	0			33	60
C. doylei							43[a]	35	**7**	**18**	**29**	**4**	0	0	0	0		74/0	20	28
C. fetus							3[a]	4	**9**	**28**	**25**	**10**	9	7	6	3[b]		13/3	23	60
C. lari							18[a]	44	**30**	**1**	**3**	**0**	0	0	0	0		30/0	35	27
Erythromycin																				
C. jejuni								0	2	45	**68**	**76**	**18**	0	0	0	0	0/1	36	77
C. coli								5[a]	17	41	**52**	**50**	**42**	2	0	0	0	0/1	25	69
C. doylei								67[a]	29	24	**6**	**2**	**0**	0	0	0	0	79/3	32	4
C. fetus								3[a]	1	14	**43**	**32**	**6**	0	0	0	0	18/3	36	68
C. lari								9[a]	3	16	**41**	**24**	**7**	0	0	0	0	24/2	33	57
Gentamicin																				
C. jejuni							0	5	27	**90**	**63**	**20**	**0**	0	0	0		0/5	43	82
C. coli							0	1	29	**103**	**59**	**12**	**0**	1	2	0		0/3	49	83
C. doylei							26[a]	42	29	**17**	**18**	**0**	**0**	0	0	0		78/0	20	17
C. fetus							7[a]	11	32	**47**	**5**	**0**	**0**	0	0	0		17/1	39	43
C. lari							4[a]	5	23	**19**	**18**	**31**	**2**	0	0	0		24/0	25	56
Meropenem																				
C. jejuni	0	0	1	**13**	**106**	**70**	20	0	0	0									50	90
C. coli	0	0	1	**9**	**22**	**47**	81	39	9	2[b]									39	37
C. doylei	6[a]	6	10	**36**	**46**	**33**	1	0	5	0								64/3	22	55
C. fetus	0	0	0	**0**	**2**	**44**	26	6	3	18[b]								18/3	37	38
C. lari	5[a]	7	4	**28**	**24**	**15**	3	4	0	12[b]								24/0	22	53

C. jejuni, C. coli, and *C. doylei* represent 210 data points per drug (5 isolates × 3 medium lots × 2 testing days × 7 labs); *C. fetus* represents 120 data points per drug (2 isolates × 3 medium lots × 2 testing days × 7 labs and 1 isolate × 3 medium lots × 2 testing days × 6 labs). *C. lani* represents 126 data points per drug (3 isolates × 3 medium lots × 2 testing days × 7 labs).

NG/CTM, No Growth/Contaminated.

[a]Results represent equal to or less than values.

[b]Results represent equal to or greater than values.

growth and the greater variability observed for *C. jejuni* subsp. *doylei*, *C. fetus*, and *C. lari* at 42°C indicates that these species should be tested at 36°C for 48 hr.

In the past few years several *in vitro* methods have been used to measure the susceptibility of *Campylobacter* to various antimicrobial agents. Disk diffusion testing is an attractive method due to its convenience and low cost. Some researchers have reported consistent results for certain drugs obtained by disk diffusion within a single laboratory.[10] However, when tested in a multilaboratory format, we found a lack of intra- and interlaboratory reproducibility, which was greater for certain antimicrobial agents (data not shown). In general, an acceptable range for a QC organism when performing disk diffusion tests is 9–12 mm, depending on the antimicrobial agent and the QC organism. In the initial studies, we conducted using the disk diffusion method for three potential QC strains of *C. jejuni*, we found that the intralaboratory variation was 12–20 mm and the interlaboratory variation was up to 30 mm, depending on the organism and the antimicrobial agent. Thus, it was not possible to correlate the disk diffusion results, by linear regression analysis, with the MIC results from agar dilution. This problem was ascribed to the peculiar growth characteristic of *Campylobacter*. This resulted in widely different interpretations of zone sizes for the same strain/antimicrobial combinations, depending on the angle and intensity of the light source, which is not seen with the dilution method. Until growth conditions are identified that eliminate ambiguity in zone end point determinations, and QC ranges have been established for this testing method, disk diffusion can not be validated for testing *Campylobacter*.

One other widely used method for antimicrobial susceptibility testing of *Camplybacter* is the epsilometer testing method (Etest, AB BIODISK, Solna, Sweden).[9,11,22] This technique is convenient and has the advantage of providing MIC values over a wide range (15 $\log_2$ dilutions). Using incubation at 36°C, it has been observed that, in general, the E-test end points fall one or more dilutions below those observed using agar dilution.[11,24] The two methods compare favorably for some drugs. Allowing for a single $\log_2$ dilution variation from the agar dilution MIC results, Ge *et al*. reported that agreement between the methods ranged from 21% for nalidixic acid to 93% agreement for gentamicin. The reported overall agreement between E-test and agar dilution for *Campylobacter* ranged from 62%[11] to 83%.[13]

The interpretation of antimicrobial susceptibility testing results for *Campylobacter* isolates is hampered by the lack of validated breakpoints for any antimicrobial agent. There are numerous reports in the literature concerning the resistance of *Campylobacter* isolates to various antimicrobial agents. The British Society for Antimicrobial Chemotherapy (BSAC) and the Comite de L'Antibiogramme de la Societe Francaise de Microbiologie (SFM), among others, have proposed interpretative criteria for organisms belonging to this genus. The BSAC has proposed resistant breakpoints of 2 μg/ml for erythromycin and 4 μg/ml for ciproflxoacin,[14] whereas the SFM has proposed 8 μg/ml for erythromycin and 4 μg/ml for ciprofloxacin.[1] These and other *Campylobacter* resistance breakpoints[4] are based largely on the population distribution of MICs, but lack clinical efficacy data. Many reports use the interpretative criteria generated for the Enterobacteriaceae, or in the case of erythromycin, those established for *Staphylococcus* spp. Because the incubation conditions required for the growth of these species are not the same as those required for *Campylobacter*, the use of these interpretative criteria for *Campylobacter* should be used with caution. There are no NCCLS breakpoints at this time for any antimicrobial agent for *Campylobacter*. The NCCLS has recently established a working group charged with trying to develop interpretive criteria for bacterial strains that may lack corporate sponsors, and *Campylobacter* have been identified as one of those organisms.

In summary, the results of this multilaboratory study standardized an agar dilution method for susceptibility testing of *Campylobacter* against five antimicrobial agents at two incubation temperatures. This study confirmed *C. jejuni* ATCC 33560 as a suitable QC strain. The availability of a standardized testing method for *Campylobacter* will provide more reliable MIC data and improve the comparison of results between testing laboratories. It will also provide a reference that may now be used to advance other susceptibility testing methods more amenable to routine laboratory use and should be the first step in establishing interpretive criteria specific to *Campylobacter*.

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